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(54) Title: METHOD OF DELIVERING THERAPEUTIC SUBSTANCES TO THE BRAIN

(57) Abstract

A method for delivering therapeutic substances to a patient's brain is disclosed. This method begins with isolating a nucleotide sequence, wherein said nucleotide sequencing encodes a protein of therapeutic value. Muscle tissue is isolated and transformed with the nucleotide sequence. The transformed muscle tissue is then implanted into the brain of a patient, whereupon said nucleotide sequence is expressed and a therapeutic substance is produced by the transformed cells.

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METHOD OF DELIVERING THERAPEUTIC SUBSTANCES TO THE BRAIN

Field Of The Invention

The present invention relates to treating disease via gene therapy. Specifically, the present invention relates to the treatment of a patient's disease through the implantation of transgenic muscle cells engineered to deliver therapeutic substances into a patient's brain.

Background

Intracerebral transplantation of genetically-modified tissue has been proposed for the treatment of several genetic and acquired neurologic disorders. Gage et al., Neurosci., 23:795-807 (1987) and U.S. Patent No. 5,082,670. These disorders include Parkinson's disease and Alzheimer's disease. The genetically-modified cells would not necessarily alleviate the neurologic symptoms by establishing synaptic, point to point connections, but would function instead by secreting therapeutic proteins, such as NGF, or metabolites, such as L-DOPA into the cerebral fluids. In addition, it may not be required that these proteins or metabolites be secreted in a regulated fashion, but instead can be expressed by unregulated, constitutive pathways that are common to all cells. Thus, many types of cells have been proposed as an intracerebral "platform" for the production of therapeutic substances.

Neuronal, glial, skin, and endocrine tissue has been transplanted into rat brain for the study of neurophysiology and for the development of new therapeutic

modalities. Gage, et al., Neuron, 6:1-12 (1991); Gash, et al., Neurobiology of Aging, 6:131-150 (1985). A critical factor in the usefulness of these transplants is the longevity of the transplanted tissue. Long-term survival
5   has been documented for fetal neuronal (Bakay, et al., Stereotact. Funct. Neurosurg. 53:1-20 (1989)) and adrenal cells (Freed et al., J. Neurosurg., 65:664-670 (1986)). Studies involving skin or glial cells are in progress (Chen et al., J. Cell. Biochem., 45:252-257 (1991),
10 LaGamma et al., Soc. Neurosci. Abstr., 17:572 (1991). Gage et al. (U.S. patent 5,082,670) discloses a method of grafting transgenic cells to treat diseases of the central nervous system. Gage et al. (column 12, line 15-50) disclose the usefulness of many different cell types as
15 choices for grafting, but do not list "muscle cells" as a choice.

        Intracranial muscle grafts have been proposed for various therapies, but the muscle grafts did not survive long-term. Heinicke, Acta Neuropathol. (Berl), 49:177-185
20 (1980); Wakai, et al. Brain Res., 386:209-222 (1986). According to Wakai, et al., only those muscle autografts placed on the surface of the brainstem survived for at least one year. In the parenchymal muscle grafts (intrabrain muscle grafts), less than 10% of the grafts'
25 volume was occupied by striated muscle cells and even this modest number of muscle fibers diminished with time. Copious, redundant basal, laminae, together with degenerated muscle cells, were common in the 1 and 3 month old parechymal grafts and the 12 month old grafts
30 contained only degenerated muscle cells and connective tissue.

        The transplantation of myoblasts into muscle has previously been proposed for the treatment of myopathies (Partridge, et al. Muscle & Nerve, 14:197-212 (1991)) and
35 metabolic disorders (Smith et al., Mol. Cell. Biol., 10:3268-3271 (1990)). Transplanted myoblasts can persist long-term in animal muscle without spontaneous tumorogenesis. Therefore, clinical trials are now exploring the safety and efficacy of myoblast

transplantation in humans (Huard et al., Clin. Sci.,
81:287-288 (1991); Law et al. Adv. Exp. Med. Biol.,
280:75-87 (1990)).

A variety of methods have been used to genetically
modify muscle cells both in vitro and in vivo by the
insertion of a foreign gene (a "transgene") into such
cells. The transgene can be expressed in a long-term
manner in these cells (Smith et al., Mol. Cell. Biol.,
10:3268-3271 (1990); Wolff et al. Science, 247:1465-1468
(1990); Yang et al. Proc. Natl. Acad. Sci. USA, 87:9568-
9572 (1990)). These manipulations are supported by a
relatively good understanding of the role and behavior of
myoblasts and satellite cells during normal muscle
development or during muscle pathology (Emerson et al.,
Molecular Biology of Muscle Development, Alan Liss, New
York, 1986; Schultz, Med. Sci. Sports. Exer., 21:S181-S186
(1989).

What is needed in the art of disease treatment is a
method of transplanting transgenic tissue into brain that
provides long-term expression of a therapeutic substance.

Summary of the Invention

The present invention is a method for delivering
therapeutic substances to a patient's brain. The method
comprises the steps of first isolating a nucleotide
sequence, wherein that nucleotide sequence encodes a
protein of therapeutic value. Muscle tissues are then
isolated and transformed with the nucleotide sequence.
These transformed muscle cells are implanted into the
brain of a patient, whereupon said nucleotide sequence is
expressed.

In a particularly advantageous embodiment of the
present invention, the transformation of the muscle tissue
is by bombarding the muscle tissues with carrier particles
coated with the nucleotide sequence.

The object of the present invention is to deliver
therapeutic molecules to a patient's brain.

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An advantage of the present invention is that a "platform" is provided for the long-term production of therapeutic molecules.

Other objects, advantages and features of the present invention will become apparent from the following specification when taken in conjunction with the accompanying drawings.

Brief Description of the Drawings

Fig. 1 is an exploded perspective view of an apparatus suitable for use in the present invention.

Fig. 2 is a top plan view of the apparatus of Fig. 1.

Brief Description of the Preferred Embodiments

The present invention relates to the use of transgenic muscle cells (myoblasts and myotubes) and muscle fiber as a "platform" for intracerebral transgene expression and the delivery of therapeutic substances to the brain by "therapeutic substance," we mean any therapeutic molecule, such as a protein or a secondary metabolite of protein activity, that will alleviate a disease state. This invention is of particular use to a patient who has a disease that can be treated by the delivery of therapeutic substances to brain cells. In brief, muscle tissue is excised from a patient. This tissue is prepared for transformation and transformed with a nucleotide sequence encoding a protein of therapeutic value. By "protein of therapeutic value" we mean that either the protein itself alleviates a disease state or produces a metabolite that alleviates a disease state. The transgenic muscle tissue is implanted in the patient's brain in a region of the brain in which the therapeutic protein is desired.

1.   Preparation of Transgenic Muscle Tissue

The examples below describe two methods of preparing suitable muscle cells. These two methods involve excising muscle tissue from the patient and either mincing the muscle before implanting or enzymatically harvesting cells

from muscle tissue. A suitable source of muscle is the patient's quadriceps muscle because a small piece of muscle may be excised without harm to the patient, but other sources could be used.

5   For the minced muscle procedure, a small piece of quadriceps muscle is removed from the patient and placed into an appropriate buffer. This muscle is minced with a sharp instrument, such as sharp-pointed scissors, into small specimens. Preferably, these specimens are

10   approximately 0.1 mm in diameter.

Enzymatic harvesting of muscles is preferably accomplished as in Yablonka-Reuveni et al. Dev. Biol., 119:252-259 (1987). Young adult or baby rats (3-5 days old) are anesthetized with 2.0 ml/100 gm body weight of

15   3.5% chloral hydrate (I.P.). The soleus muscle is removed using sterile technique and placed in a 60 mm sterile petri dish with a small volume of CMF Hank's solution (1X, Gibco). Muscle is rinsed with Hank's solution to remove any blood and then minced finely with sterile scissors.

20   The minced muscle is then transferred to a 50 ml flask containing the muscle enzyme solution (0.169% trypsin, 0.085% collagenase, CMF Hanks, pH 7.4) that has been pre-warmed for 15 min at 37 °C and incubated for 55 min (for baby rat muscle, incubation time could be 20 min)

25   while the flask is shaken every 15 min and the pH checked. Sterile 0.1 M NaOH is used to regain proper pH color when necessary. Contents of the flask are transferred to a plastic sterile test tube and centrifuged for 5 min at "4" setting on the clinical centrifuge. After 2 washings with

30   complete media, NRM (normal rat media, 5% chick embryo extract <Gibco, BRL>, 15% horse serum, 80% modified Eagle's media), the pellets are resuspended in 2.5 ml NRM and mechanically dissociated through an 18-gauge needle. The resulting cell suspension is filtered through a nitex

35   filter to eliminate myotubes and connective tissue, and then purified by Percoll (Sigma) gradient centrifugation. The muscle cell fraction is manually collected, diluted with NRM, and centrifuged in the clinical centrifuge for 10 min. After the resulting pellet is resuspended in an

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appropriate volume of NRM, and the cell number is counted, the cells are plated out into 35 mm culture dishes (10^6 cells per dish). The cells are then maintained in an incubator (37 °C, humidified atmosphere of 5% CO_2 in air)

5   with NRM media that is changed every other day.

The invention is directed toward the introduction of exogenous, typically chimeric, genetic constructions into muscle cells. Such exogenous genetic constructions consist of DNA from another organism, whether of the same

10   or different species. The exogenous DNA construction would normally include a coding sequence for a transcription product or a protein of interest, together with flanking regulatory sequences effective to cause the expression of the protein in the transformed cells of an

15   organism. Examples of flanking regulatory sequences are a promoter sequence sufficient to initiate transcription and a terminator sequence sufficient to terminate the gene product, whether by termination of transcription or translation. Suitable transcriptional or translational

20   enhancers can be included in the exogenous gene construct to further assist the efficiency of the overall transformation process and expression of the protein result in the transformed muscle cells. A signal sequence, effective to cause secretion of the therapeutic

25   protein into the intracellular space, might usefully be linked to the protein. The inserted construction could itself be RNA, as an alternative to DNA, if only transient expression of the gene product is desired.

In many applications, it is most advantageous if

30   secretion of the therapeutic protein is achieved in transgenic cells. This can be accomplished by attaching to the 5' end of the coding region for the protein of interest a signal peptide coding sequence. The signal peptide should preferably condition extra-cellular

35   transport and cleavage so as to deliver the protein into the intracranial space.

The excised and treated muscle cells are then transformed with the exogenous nucleic acid construction. The art of mammalian cell transformation contains many

suitable methods. The examples below describe muscle cells transformed by injection with a DNA-containing solution, by a Lipofectin-mediated method and the transformation of muscle cells via bombardment with
5 DNA-coated particles.

A suitable transformation method would produce enough transformed cells so that detection of the transformants is reasonably efficient. A suitable method would not damage the muscle platform cells to the extent that at
10 least a portion of the cells could not be implanted and produce the desired protein.

Because the transformation event may not be highly efficient, it may be necessary to select or screen for muscle cells have been transformed. This objective may be
15 accomplished with the use of suitable marker or selectable agent genes transformed into the platform muscle cells. These genes may be linked to the gene of therapeutic interest or may simply be co-transformed.

A marker gene encodes a gene product which can be
20 easily assayed, such as beta-galactosidase. The presence of the product of the marker gene indicates that the cell is transformed. Even if the assay destroys the examined cells, information as to the percentage of transformants would be gained.

25 A selectable marker gene encodes a product which the cell would need to survive in a specific environment. An example of a selection gene would be a gene encoding antibiotic resistance. Therefore, the cells in question could be subjected to the antibiotic and only the
30 transformed cells would survive.

2. Preparation of Patients

Once transformed muscle tissue has been obtained, the tissue must be implanted in the patient's brain. Preferably, a patient's own muscle will be used for brain
35 implantation to avoid immune rejection, i.e. the transplant is autologous. Surgical techniques that are known in the art will be used to create a skull window in the section of the patient's brain into which the

transgenic implant is desired and the transgenic tissue
will be implanted.

EXAMPLES

1. "Minced" Muscle Implants.

A. Preparation of Muscle

A 5 X 5 X 4 mm piece of a quadriceps muscle was
removed from one- to two-month-old Lewis or Sprague Dawley
rats and placed into phosphate buffered saline (PBS)
containing 0.6% glucose at room temperature. Blood was
removed by washing with this solution. The muscle was
minced with a sharp-pointed scissors (Fisher Scientific)
into specimens of 0.1 mm in diameter.

B. Preparation of Animals

The same animal served as a host for brain
implantation of its minced muscle to avoid immune
rejection. A 2.5 mm diameter skull window was opened in
the rat using a dental drill. The window was 2 mm lateral
to the bregma over the frontal lobe. The dura was
carefully opened and a piece of the brain tissue was
aspirated to make a cavity that was 2 mm in diameter and 2
to 3 mm deep. After application of gel foam (The Upjohn
Co.) to sop the bleeding, the minced muscle was implanted
with an 18 gauge metal cannula. The overlying skin was
closed with subcutaneous sutures and clips.

C. Analysis of Implants

Four to six animals with minced muscle implants were
sacrificed at one week, one month, two months, four months
and six months to determine if the intrabrain muscle graft
could survive long-term. Hematoxylin and eosin (H&E)
staining of the brain sections revealed that muscle grafts
were present at each of these time points.

From examining the stained grafts, we determined that
the one-week-old muscle grafts were comprised of myotubes
or small myofibers in approximately equal proportion. Few
necrotic fibers were evident at this time. The two-

week-old grafts were mostly comprised of myofibers with a few myotubes at the periphery of the grafts. The grafts older than two weeks were comprised mostly of myofibers with striations. Thus, the transplanted cells were developing in a normal manner.

The progression from myotubes to myofibers in the brain grafts were similar to the progression observed when minced muscle was grafted into muscle (Grounds et al. J. Pathology, 132:325-341 (1980); Partridge et al. J. Neurol. Sci., 33:425-435 (1977)). However, the rate of the progression to myotubes and myofibers now observed in the adult muscle to brain grafts appeared faster than what was previously observed in adult muscle to muscle grafts. This rapid progression was more like that observed when neonatal muscle was grafted into muscle (Grounds et al., supra).

Fewer than ten percent of the myofibers in the grafts older than two weeks contained central nuclei. The rest of the myofibers contained peripheral nuclei. The large percentage of peripheral nucleation found in the myofibers is an indication of well-differentiated muscle cells. This peripheral nucleation was an unexpected finding because previous studies had indicated that grafts of minced, adult muscle grafted back into muscle contained an increased percentage of central nuclei (Grounds et al. supra). In fact, adult rodent muscle that has undergone regeneration under a variety of conditions typically contained myofibers with central nuclei. In contrast, minced neonatal muscle or neonatal myoblasts formed myofibers with peripheral nuclei when transplanted into muscle (Grounds et al., supra). Both the preponderance of myotubes at one week and the peripheral nucleation in the adult muscle to brain grafts were similar to the behavior of neonatal muscle cells. These observations suggest that possibility that the host brain may have provided factors that enabled the transplanted adult muscle to form myotubes more quickly and peripheral nucleated myofibers.

Brains that were similarly implanted with slivered but not minced muscle contained dying myofibers at one

week and a few myotubes at two weeks. Mincing may enable
better vascularization of the grafts by disrupting the
epimysium and perimysium.

5   All of the four brains for each time period contained
substantial grafts. Measurement of the graft maximum
diameter provided a semi-quantitative assessment of their
size. The mean maximum diameters ($\pm$ indicates standard
error) of the grafts did not change over time and were
1.94 ($\pm$ 0.15) mm at one week (n=4), 1.78 ($\pm$ 0.11) mm at
10   two weeks (n=6), 2.17 ($\pm$ 0.22) mm at one month (n=5), 1.77
($\pm$ 0.10) mm at two months (n=6), 2.33 ($\pm$ 0.34) mm at four
months (n=4), and 2.13 ($\pm$ 0.21) mm at six months (n=4)
after transplantation. These results indicate that the
muscle grafts were present in the brains for at least six
15   months. All of the experimental animals survived without
any obvious adverse effect.

           The grafted, minced muscles were further studied by
immunohistochemically staining of the brains for muscle
myosin using MF20 anti-myosin antibody (Developmental
20   Studies Hybridoma Bank, Baltimore, MD) and a
FITC-conjugated, anti-mouse, IgG antibody (Sigma) as
previously reported in Bader, et al. J. Cell Biol.,
95:763-770 (1982). Only brains implanted with minced
muscles contained cells with myosin staining in a striated
25   pattern. Brains transplanted with unminced muscles had
much less myosin staining.

           We examined the grafts via electron microscopy. This
technique showed that the muscle grafts contained normal
myofilaments six months after transplantation.

30   In summary, the presence of striations and peripheral
nuclei in six-month-old muscle grafts suggested that
minced muscles survived long-term in a healt￼ and
differentiated state.

           D.   Implantation of Transgenic Muscle ᵢ l

35   We transplanted muscle cells that had been
genetically modified by the intramuscular injection of
plasmid DNA. Previous studies had shown that muscle cells

in *vivo* can take up and stably express plasmid DNA
delivered to the extracellular space.

Quadriceps muscles were injected intramuscularly with
200 μg of the luciferase expression plasmid, pRSVL (De Wet
et al., *Proc. Natl. Acad. Sci. USA*, 82:7870-7873 (1985))
as previously reported in Acsadi *et al.*, *New Biologist*
3:71-81 (1991) and Wolff, *et al.* *Science*, 247:1465-1468
(1990). Four days after injection, the muscles were
either analyzed directly for luciferase activity or
implanted into the brain. Extracts of the quadriceps
muscles or the frontal part of the recipient brains were
prepared in 200 μl of lysis buffer and 20 μl of the
supernatant was assayed for luciferase as previously
reported in Acsadi *et al.* *New Biologist*, 3:71-81 (1991).
The muscle extracts prior to transplantation contained a
mean of 1,086,460 ($\pm$ 206,729) Light Units ("L.U.") (n=5).
At one day after transplantation, the recipient brains
contained 70,697 ($\pm$ 68,591) L.U. (n=4). The recipient
brains contained only background levels of luciferase
(<400 L.U., n=12) at seven days post-transplantation.

2.   **Enzymatically Harvested Muscle Implants**.

A.   **Preparation of Myoblasts**

Myoblasts were enzymatically harvested from one to
three-day-old Lewis rats and purified by Percoll (Sigma)
gradient centrifugation as in Yablonka-Reuveni *et al.*,
Dev. Biol., 119:252-259 (1987) as described above. The
confluent myotubes in a 35-mm plate were harvested without
trypsinization using a rubber policeman.

B.   **Implantation of Cells**.

The muscle tissue was either implanted into the
cortex or injected stereotaxically into the caudate
nucleus of one- to two-month-old Lewis rats.

Adult male Lewis rats (200-300 gm) were used as
recipient animals. The rats were anesthetized with
intramuscular injection of a mixture of Ketamine (110
mg/kg) and placed securely in the stereotaxic instrument.

Intracaudate implantation consisted of the following:
Myoblast or myotube suspensions were stereotaxically
injected into the striatum of host rat brain at the
following coordinates (in mm) relative to the bregma and
the dural surface, and with the tooth-bar set at zero:
A=0.2-0.7, L=2.5-3.0, V=5.5-6.5 (Paxinos and Watson, The
Rat Brain in Stereotaxic Coordinates, 1982) The cell
suspension was centrifuged and the pellet was resuspended
in a volume of Basic Medium to yield approximately 40,000
vital cells per microliter. A total of 10 μl of the cell
suspension (equivalent to a total of approximately 50 X
10^4 viable cells) was delivered to the striatum by a 50 μl
Hamilton syringe with a 23 gauge needle over a period of 5
min. After each injection to the striatum, the needle was
left in place for five min and slowly withdrawn from the
brain. A small piece of gelfoam was inserted into the
opening in the skull, and the scalp sutured.

Measurement of the maximum diameter of the graft
provided a semi-quantitative assessment of the size of the
graft over time. In the implanted group, the mean maximum
diameters (± indicates standard error) of the grafts were
1.36 (± 0.08) mm at one week (n=6), 1.60 (± 0.11) mm at
two weeks (n=6), 1.33 (± 0.20) mm at one month (n=6), and
1.28 (± 0.19) mm at six months (n=6) after
transplantation.

Myosin staining (Bader, et al., J. Cell. Biol.,
95:763-770 (1982)) and H&E staining of the grafts six
months post-implantation confirmed that the muscle cells
contained myosin and myofilaments. Brains injected with
myotube cultures into the caudate nucleus also contained
myosin-positive muscle cells after six months.

Electron microscopy revealed intact, nucleated muscle
cells with myofilaments.

C.   Implanting Transgenic Muscle Cells.

A method of transfecting myotubes in culture via
liposome-mediated transformation was developed. Myotubes
in a 35-mm plate, differentiated for 5 to 8 days, were
transfected using Lipofectin as in Felgner et al., Proc

Natl. Acad. Sci. USA, 84:7413-7417 (1987). The myotube
cultures were washed three times with serum-free Opti-MEM
(BRL). After exposing the cultures to pre-mixed complexes
of 15 μg of pRSVL and 45 μg of Lipofectin in 1.0-ml of
Opti-MEM for four hours, 1.5-ml of complete medium was
added. Three days post-transfection, the cultures
contained 11,370,000 (± 1,598,376) L.U. of luciferase
activity (n=6) which is approximately five to ten times
greater than the activity obtained with 3T3 mouse
fibroblasts transfected using similar methods. The
myotubes were similarly transfected with the _E. coli_
beta-galactosidase plasmid, pRSVLac-Z (Norton _et al_. _Mol._
Cell. Biol., 5:281-290 (1985)) to determine the percent of
myotubes transfected. Approximately 50% of the myotubes
transfected with 15 μg of pRSVLac-Z and 45 μg of
Lipofectin were stained blue in this assay, thus
indicating beta-galactosidase expression.

The transfected myotubes were implanted into adult
Lewis rat brains. The sections of brains grafted with
myotubes transfected with pRSVLac-Z were stained
histochemically for beta-galactosidase activity as
described in Acsadi, _et al_. _New Biologist_, 3:71-81 (1991).
The muscle grafts contained beta-galactosidase-positive
myofibers two weeks post-transplantation. More
quantitative information was obtained using
pRSVL-transfected myotubes. After implantation of pRSVL-
transfected myotubes, mean luciferase activities (±
standard errors, n=6 for each time period) were 167,142 (±
71,626) L.U. at seven days post-transplantation, 195,013
(± 70,697) L.U. at two weeks post transplantation, 150,537
L.U. (± 27,500) at four weeks, and 191,852 L.U. (± 33,233)
at eight weeks post-transplantation. The contralateral
side of the brain that did not receive implants contained
background levels of luciferase (<400 L.U.).

Although luciferase expression decreased
substantially from the pre-implantation levels of
11,370,000 L.U. to 167,142 L.U. seven days after
transplantation, expression was stable from seven days
through two months post-transplantation. The reason for

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the large drop in luciferase expression soon after
transplantation is probably due to damage to the myotubes
during the transplantation process.

In summary, we established that grafted muscle cells
5   survived in the brain for at least six months and that
plasmid expression was stable for at least two months.
The robust and consistent long-term survival of the trans-
planted muscle cells suggests that this cell type may be a
useful "platform" for transgene expression in brains. Our
10   experiments indicate that differentiated myoblast cultures
transfected with plasmids can stably express a transgene
in the brain for at least two months.

3.   Particle-mediated Transformation

In a particle-mediated transformation method, cells
15   or tissue are bombarded by nucleic acid-coated particles.
The coated particles deliver foreign genetic material to
the interior of the cell. The particle-mediated
transformation procedure described in published PCT
application WO 91/00359 is especially suitable for
20   creating transgenic mammalian cells. This technique could
be used to create transgenic muscle cells which could then
be implanted in brain, as described above. Suitable
muscle cells could be obtained as described above. The
particle bombardment could occur either before or after
25   the mincing or enzymatic digestion used to isolate the
muscle tissue.

There are other particle-mediated transformation
methods that could be equally suitable. One suitable
particle-mediated transformation is described in PCT
30   published application WO 91/07487. The apparatus used in
this technique is commercially available from BioRad.

The following describes the transformation of muscle
cells by the method of published PCT application WO
91/00359: Figs. 1 and 2 illustrate an apparatus suitable
35   for particle-mediated transformation of muscle cells and
tissues. The present invention makes particular use of
the apparatus of Figs. 1 and 2 providing an adjustable
electric discharge to physically accelerate DNA coated

onto small particles into the genetic material of muscle
cells. The apparatus consists of a spark discharge
chamber 12 into which are inserted two electrodes 14 which
are spaced apart by a distance of approximately 1 - 2 mm.
5   The spark discharge chamber is a horizontally extended
rectangle having two openings 16 and 18 out its upward
end. One opening 18 is covered by an access plate 20.
The other opening, located opposite from the electrodes
14, is intended to be covered by a carrier sheet 22. The
10   electrodes 14 are connected to a suitable adjustable
source of electric discharge voltage. Such a source of
electric discharge voltage would preferably include
suitable electric switching connected to a capacitor of
the 1 to 2 microfarad size range. The amount of the
15   voltage of the charge introduced into the capacitor is
adjustable, such as through the use of an autotransformer,
through a range of, for example, 1 to 50,000 volts.
Suitable switching is provided so that the capacitor can
be discharged through the electrodes 14 safely and
20   conveniently by a user.

The carrier sheet 22 is intended to be place upon the
opening 18 on the spark discharge chamber 12. Carrier
sheet 22 is preferably a sheet of aluminized saran coated
mylar. Approximately 5 - 10 millimeters above the opening
25   in the discharge chamber is a retaining screen 24. Placed
approximately 5 - 25 millimeters above the retaining
screen is a target surface 26.

An exogenous foreign gene construct intended to be
transformed into the muscle cells is prepared by suitable
30   DNA or RNA preparation techniques well known to one of
ordinary skill in the art. The nucleic acid is dried onto
small particles of a durable dense material such as gold,
the particles typically being 1 to 3 microns in size. One
suitable method of drying the nucleic acid onto the gold
35   particles is described in PCT application WO 91/00359. In
brief, the DNA is applied to the particles after precipi-
tation with 25 mM spermidine/6% polyethylene glycol (M.W.
3000) with the addition of $CaCl_2$ to a final concentration
of 0.6 M. Preferably, the DNA is coated onto the beads at

a concentration of 0.1-0.5 μg DNA per Mg particles. The
coated particles are rinsed in 100% ethanol and applied to
the carrier sheet 22.

5   The coated carrier particles are then placed upon the
carrier sheet 22 which is inserted on top of the spark
discharge chamber 12. A target tissue, such as muscle
cells or tissue, is then placed adjacent to the target
surface 26. Then a small droplet of water, approximately
2 - 4 microliters in size, is placed bridging between the
10   ends of the electrodes 14. The access plate cover 20 is
then placed over the top of the discharge chamber 12. The
atmosphere between the carrier sheet 22 and the target is
replaced with helium, by enclosing the apparatus and
target and introducing helium in the enclosure in
15   sufficient quantity to largely displace the atmospheric
gases.

          Appropriate electronic switching may be used to
initiate a spark discharge between the electrodes.
Preferably, the apparatus is operated at a spark discharge
20   level of 5-15 kV. The force of the electric discharge
bridges the spark discharge gap between the electrodes 14
instantly vaporizing the small droplet of water placed
there. The force of the vaporization of that water
creates a shock wave within the spark discharge chamber 12
25   which radiates outward in all directions. The impact of
the shock wave upon the carrier sheet 22 propels the
carrier sheet 22 upwards with great velocity. The
traveling carrier sheet 22 accelerates upward in direction
until contacting the retaining screen 24. The presence of
30   the helium provides less drag on the flight of the carrier
sheet as well as less force for the shock wave to
propagate to the target. The carrier sheet 22 is retained
at the retaining screen 24, and the DNA-coated particles
previously applied to the sheet 22 fly off of the carrier
35   sheet and travel freely on toward the target surface. The
particles proceed into the target surface and enter the
muscle cells thereon. The momentum of the particles as
they impact the surface of the target tissue is adjustable
based on the voltage of the initial electric discharge

applied to the electrodes 14. Thus by variations in the amount of electric energy discharged through the electrodes 14, the velocity by which the particles impact the target can be adjusted, and thus the depth of penetration of the particles into the tissue of a target, can be continuously adjusted over the range of adjustment of the electric discharge throughout the electrodes 14.

Implantation and analysis of the implanted transgenic muscle cells are preferably performed as described above. Previous experience with particle-mediated transformation in a variety of cell types has revealed that in most, if not all, cell types a significant level of post-blasting transient gene activity is obtained followed by a decrease in expression to a lower but longer term level of expression. Some of the treated cells become permanently transformed. Thus once the muscle platform cells become capable of long-term survival in the brain, long-term expression of the transgene and delivery to the brain can also be achieved.

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CLAIMS

We Claim

1.   A method for delivering therapeutic substances to a patient's brain, comprising the steps of:
5   (a)   constructing a nucleotide sequence, wherein said nucleotide sequence is capable of expressing a protein of therapeutic value in the patient's cells;
          (b)   isolating muscle tissue from the patient;
          (c)   transforming cells of said muscle tissue with
10   said nucleotide sequence; and
          (d)   implanting said muscle tissue into the brain of a patient, whereupon the protein is expressed by the nucleotide sequence and a therapeutic substance is produced by the transformed cells.

15   2.   The method of claim 1 wherein the transforming of step (c) is by bombarding the muscle tissue of step (b) with carrier particles coated with the nucleotide sequence of step (a)

          3.   The method of claim 1 wherein the transforming
20   of step (c) is by liposome-mediated transformation.

          4.   The method of claim 1 wherein the transformation of step (c) is by injection of a nucleic acid-containing solution into muscle tissue.

          5.   The method of claim 1 wherein the isolated
25   muscle tissue of step (b) is obtained by mincing muscle.

6.   The method of claim 1 wherein the isolated
muscle tissue is obtained by enzymatically digesting
muscle.

7.   The method of claim 1 wherein the implanting of
step (d) is into the cortex of the brain.

8.   The method of claim 1 wherein the implanting of
step (d) is into the caudate nucleus of the brain.

9.   The method of claim 1 wherein the patient is a
human.

10.   The method of claim 1 wherein the isolated
muscle tissue is myoblast cells.

11.   The method of claim 10 additionally containing
the step of culturing the myoblast cells until some of the
myoblasts have formed myotubes and wherein the culturing
takes place before the transformation of step (c).

12.   A method for delivering therapeutic substances
to a patient's brain, comprising the steps of:
    (a)   constructing a nucleotide sequence, wherein said
nucleotide sequence is capable of expressing a protein of
therapeutic value in the patient's cells;
    (b)   coating the nucleotide sequence onto carrier
particles;
    (c)   excising muscle tissue from a patient;
    (d)   accelerating the coated carrier particles into
the muscle tissue so as to transform some of the cells in
the muscle tissue with the nucleotide sequence, and

    (e)   implanting the muscle tissue onto the brain of the patient at a location at which the therapeutic substance is desired to be present.
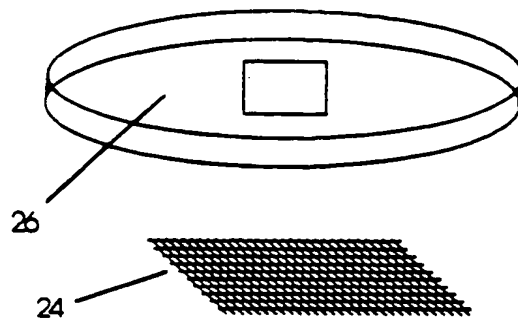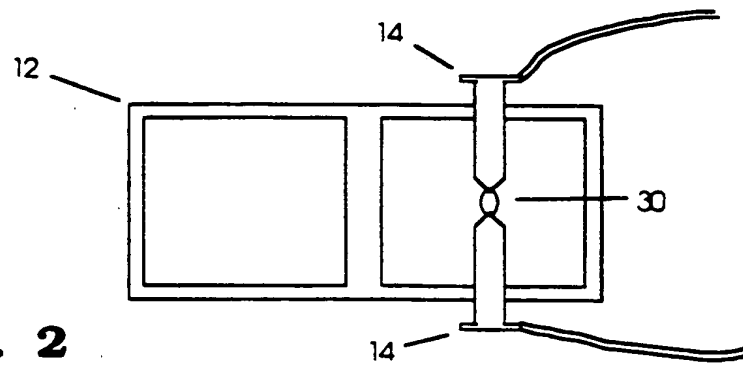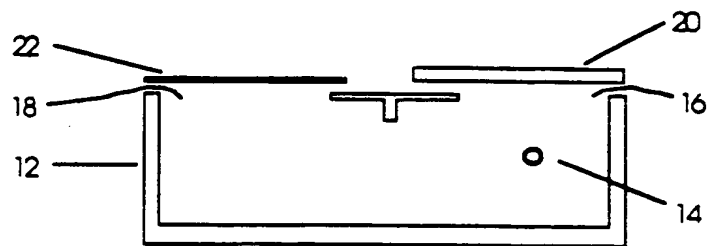
FIG. 1

FIG. 2

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5)   :IPC(5):   A61K 37/00
US CL   :U.S.CL: 424/93
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   U.S.CL: 424/93

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, "transplatation, ""muscle", "brain", author search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Acta Neuropathologica, (Berl), volume 49, no. 3, issued 1980, Heinicke, "Vascular permeability and axonal regeneration in tissues autotransplanted into the brain", pages 175-185, see entire article.	1, 5-8, 10, 11, 12
Y	Molecular and Cellular Biolog, volume 10, no. 6, issued June 1990, Smith et al., "Genes transferred by retroviral vectors into normal and mutant myoblasts in primary cultures are expressed in myotubes", pages 3268-3271, see entire document.	1, 5-8, 10, 11, 12

[X] Further documents are listed in the continuation of Box C.   [ ]   See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 MAY 1993	0 8 JUN 1993

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer SUZANNE ZISKA
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Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, volume 254, issued 06 December 1991, Barr et al., "Systemic delivery of recombinant proteins by genetically modified fibroblasts", pages 1507-1509, see entire document.	9
Y	WO,A, 91/00359 (Brill et al) 10 January 1991, see entire article.	2, 12
Y	Science, volume 247, issued 23 March 1990, Wolff et al., "Direct gene transfer into mouse muscle in vivo", pages 1465-1468, see entire article.	4
Y	Proceedings of the National Academy of Sciences, volume 84, issued November 1987, Felgner et al., "Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure", pages 7413-7417, see entire article.	3